

Current Biology

Sequential Molt in a Feathered Dinosaur and Implications for Early Paravian Ecology and Locomotion

Highlights

- Among Aves, molt strategy is correlated with habitat selection and flight ability
- An ancestral feather molt strategy for Aves is probably sequential
- *Microraptor* is the earliest known feathered vertebrate with a sequential wing molt
- *Microraptor* likely maintained its flight ability throughout the entire year

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In Brief

Kiat et al. report the first fossilized evidence of sequential wing feather molt, which was identified in the Early Cretaceous four-winged dromaeosaurid *Microraptor*. The study concludes that the development of sequential molt is the outcome of evolutionary forces to maintain flight capability throughout the entire annual cycle.



Report

Sequential Molt in a Feathered Dinosaur and Implications for Early Paravian Ecology and Locomotion

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SUMMARY

Feather molt is an important life-history process in birds, but little is known about its evolutionary history. Here, we report on the first fossilized evidence of sequential wing feather molt, a common strategy among extant birds, identified in the Early Cretaceous four-winged dromaeosaurid *Microraptor*. Analysis of wing feather molt patterns and ecological properties in extant birds imply that *Microraptor* maintained its flight ability throughout the entire annual cycle, including the molt period. Therefore, we conclude that flight was essential for either its daily foraging or escaping from predators. Our findings propose that the development of sequential molt is the outcome of evolutionary forces to maintain flight capability throughout the entire annual cycle in both extant birds and non-avian paravian dinosaurs from 120 mya.

RESULTS AND DISCUSSION

Recent advances in our understanding of the origin of key avian attributes, particularly the origin of feathers [1], wings [2], and other important structures [3, 4], have greatly benefited from an integration of paleontological and neontological data [5]. This has led to important paleoecological and functional insights regarding the evolution of these structures near the origin of birds [6]. Here, we apply this approach to understand the evolution of feather molt, an important but understudied avian feature. Feather molt is essential for birds because old feathers become worn and their various essential functions, such as thermoregulation, visual communication, camouflage, and particularly aerodynamic performance, deteriorate over time [7]. To maintain the morphological integrity of the plumage to preserve function, feathers must be renewed through the process of molting. Previous studies indicated that different species of modern birds vary in the molt strategy of their wing feathers [8, 9]. These strategies are (1) sequential molt that includes a gradual and directional replacement of the flight feathers that are usually slow and symmetrical between the two wings, (2) simultaneous replacement of all the flight feathers (simultaneous molt), and (3) a gradual, but not ordered, molt, in which feather replacement has no predictable sequence or direction (irregular molt). Due to lack of information regarding the distinction between simultaneous and irregular molt among many species, we used in this study only two strategies: “sequential molt” and “non-sequential molt”,

the latter including both simultaneous and irregular molts. Different wing molt strategies affect flight performance to varying degrees through their differing morphological deficits to the wing surface area and the duration of the molt process until its completion [10, 11]. Therefore, bird wing feather molt strategy has important implications for bird ecology, including movement-related foraging behavior, prey capture success, and escape from predation [12–14].

In order to uncover the ecological and functional implications of feather molt and to investigate how molt strategies first evolved, we collected wing molt data from a total of 302 extant bird species. For these species, we collected data from 51 flightless species, 61 bird species that are flightless only during the molting period, and 190 flying species that maintain their flight abilities throughout their entire post-fledging adulthood, even during molting (Table S1).

An ancestral reconstruction trait analysis of these 302 living species, set in the framework of a recently published comprehensive phylogeny of modern birds [15], indicates that the ancestral molt strategy for modern birds (Neoaves) is sequential (99.98%; continuous-time Markov chain). This is likely also the ancestral condition for all Aves (97.42%; Figures 1 and 2). Therefore, non-sequential molts (including both irregular and simultaneous) are derived within Aves. Our analysis agrees with previous studies [8, 16] and demonstrates a strong correlation between molt strategies and flight capabilities: sequential molt is found only in flying species that maintain their flight abilities



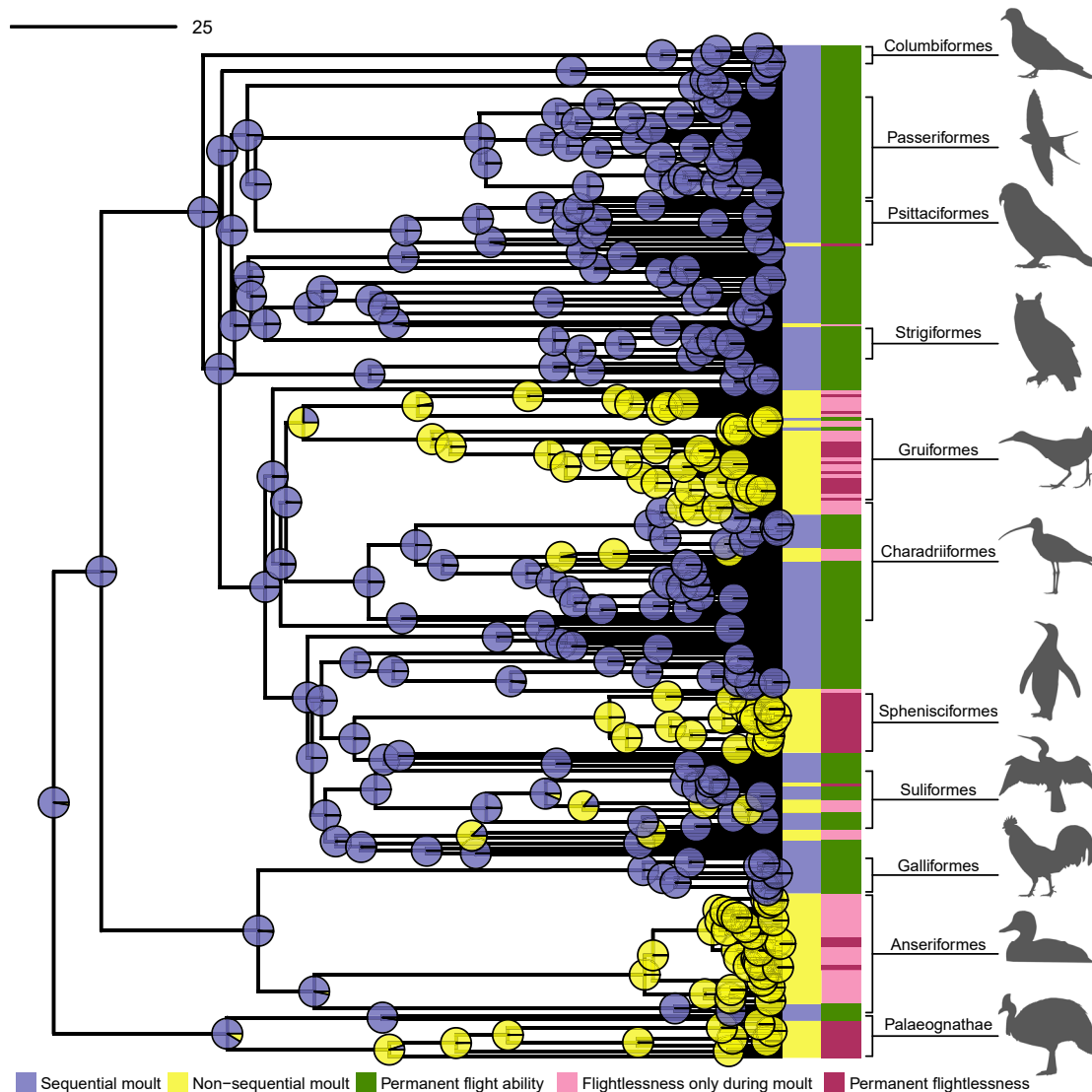


Figure 1. The Relationship between Flight (Permanent Flightlessness, Temporary Flightlessness Only during Molting, and Flight throughout the Entire Annual Cycle, Including during the Molting Period) and Molt Strategy in Extant Bird Species

Ancestral trait reconstruction analysis (302 species; continuous-time Markov chain) using a comprehensive phylogeny of modern birds [15]. Our results indicate that the ancestral molt strategy of Aves is sequential and that the transition between sequential molt to a non-sequential molt strategy occurred independently at least 10 times in the history of this group. The scale bar (top left) represents 25 Ma.

during the molting period, irregular molt is present only in flightless birds, and simultaneous molt is present in both flightless birds and flying species that are unable to fly only during the molting period. There is not a single flightless bird species that molts its primary feathers sequentially, probably because, unlike flying birds, in these species, the evolution of feather molting was not shaped by flight-performance-related factors. Two flightless birds species that relatively recently evolved from flying taxa (the Flightless Cormorant *Nannopterum harrisi* and the Kakapo *Strigops habroptilus*) both show irregular molts; the concomitant loss of their sequential molt and flight capability indicates that there is a cost to maintaining a sequential molt, such that when aerodynamic-related selection pressures are relaxed due to flightlessness, sequential molt is not maintained. The molt

strategy is thus a strong predictor of flight ability in extant birds, and more specifically, a sequential molt strategy indicates unambiguously that flight abilities are retained during the molting period. Hence, maintaining flight ability during molting is likely essential for the survival of most volant bird species, for which movement by flight is critical for their foraging and escape from predators [8, 17].

As suggested by previous authors [8, 16–18], our data also indicate a correlation between molt strategies and habitat selection. Sequential molt occurs only among species in which flight is essential for foraging or whose habitat during the molting period does not provide protection from predation. In contrast, non-sequential molt (including both irregular and simultaneous molt) takes place among volant species whose habitat is

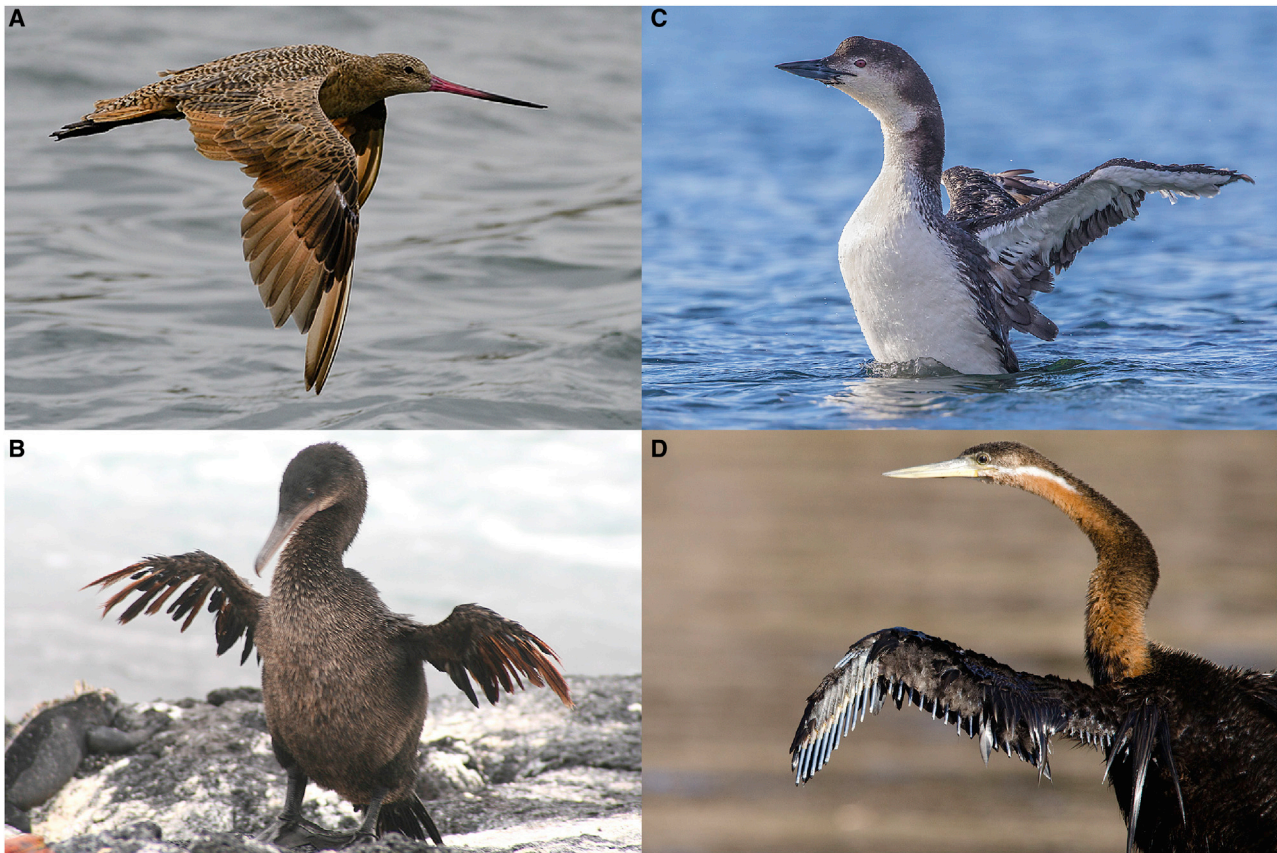


Figure 2. Examples of Two Molt Strategies: (1) Sequential Molt and (2) Non-sequential Molt

(A) Marbled godwit *Limosa fedoa* during a sequential molt that includes gradual and directional replacement of the wing's flight feathers, starting from the innermost primary and moving outward toward the wing's distal tip (credit: G. Gartner).

(B) Flightless cormorant *Nannopterum harrisi* during a non-sequential molt that includes an irregular, gradual replacement of its flight feathers; both wings present a mixture of old, new, and growing feathers without any order or symmetry between the two wings (credit: T. Salmond).

(C) Common loon *Gavia immer* after simultaneous shedding of all the wing's flight feathers as part of its non-sequential simultaneous molt (credit: S.P. d'Entremont).

(D) African darter *Anhinga rufa* that is characterized by a non-sequential molt, in which its flight feathers grow simultaneously (credit: L. Francey). The results of our analysis indicate a strong relationship between molt strategy and bird life history, flight ability, and habitat selection during the molting period. Non-sequential molt occurs among flightless species or in species that molt within protected habitats (e.g., marshes or lakes), while sequential molt takes place among flying species that inhabit habitats that do not provide protection from predation.

characterized by abundant food resources that do not require flight for food consumption and that provides protection from predation. This strategy occurs in aquatic birds, which can avoid predation by diving (e.g., seaducks, grebes, and loons), and species that spend the molting period within a very densely vegetated habitat (e.g., marshes, grasslands, or islands without predators) that provides sufficient safety from predators while offering high abundance of food that can be easily consumed while the bird is temporarily flightless [17].

In addition to the information regarding the molt strategies of extant birds, we found evidence of active feather molting in a specimen of the volant dromaeosaurid *Microaptor* (IVPP V13352) housed in the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP), Beijing, China [19]. In this specimen, a distinct gap is visible in the right wing. We propose that the gap, which is located between P(c) and P(g), constitutes a wing molt gap created by three growing primary feathers, P(d)–P(f), which are much shorter than the remaining primaries

(Figure 3A). Because P(f) is at an advanced growth stage compared with P(e), and P(e) is at an advanced growth stage compared with P(d), the growing primary feathers exhibit a clear sequential molt strategy that most likely began with P(g), the innermost primary identified in the fossil, and moved outward toward the wing's distal tip, as in many extant flying birds (Figure 3). The wing feathers P(a)–P(g) are identified as primaries based on their location, size, and morphology (Figures 3 and S1). The shorter feathers, P(d) and P(e), are identified as growing primaries and not as wing coverts, mainly due to their location within the set of the longer primaries, between P(c) and P(f) (Figures 3 and S1). Furthermore, the width of these feathers is similar to that of the longer primary feathers, P(f) and P(g). We identify these short feathers as growing primaries and not feathers whose preservation is incomplete based on the well-preserved morphology of their distal apices (Figures 3 and S1). Visual inspection of the rachis and the barbs allowed us to identify the outline of each feather and the sequence of the feather in the

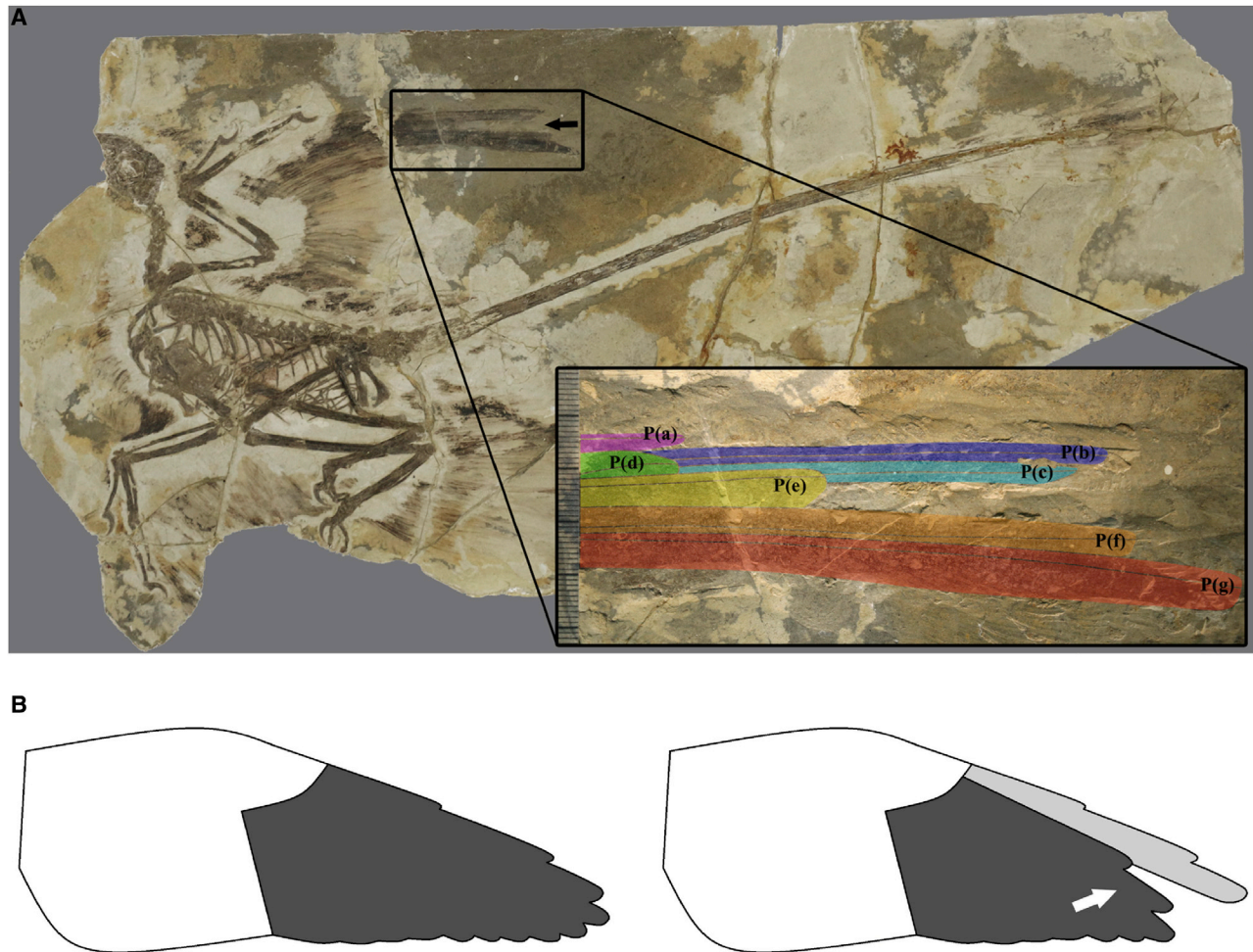


Figure 3. Wing Molt in *Microraptor* (IVPP V-13352)

(A) This specimen exhibits an active molt in the primaries (the arrow points to the molt-related wing gap). The bottom right inset shows how we identified seven primaries in the specimen's wing, marked as P(a)–P(g). Based on the molt sequence (see also Figure S1), one may infer that the long inner primaries are new, the shorter primaries in the mid-hand are growing, and the long outermost primaries are old.

(B) Illustrations of wing shape in *Microraptor*, where all primary feathers are fully grown (left) and during active wing molting of the primary feathers (right). The dark gray feathers are new (fully grown or actively growing), and the light gray ones are old and unmolted feathers (the arrow indicates the direction of molt sequence).

wing, as shown in Figures 3 and S1. In addition, the newer feathers, P(d)–P(g), are darker, wider, and more rounded than the older ones, P(a)–P(c), which resemble the differences between new and old feathers in extant birds (for example, Figure 2A). Because most of the left wing feathers are missing (Figure 3), molt symmetry cannot be determined. Notably, no such gap in the wing is observed in the other *Microraptor* specimen (BMNH PH-881) [20] that we inspected.

Although molting has been identified in juvenile enantiornithines fossils (Avialae) from the Early Cretaceous [21], this discovery of feather molt in *Microraptor* provides the first clear evidence of sequential wing feather molt in the fossil record and the first identification of molting in non-avian dinosaurs. Our ancestral reconstruction trait analysis suggests that the sequential molt strategy was present in the ancestor of crown birds, which existed at least 70 mya (Figure 1). *Microraptor* is known from a deposit dated to 120 mya [22–24]. Thus, this report extends the documented evolutionary history of sequential feather

molting back by up to 50 Ma, prior to the origin of crown birds, as has been suggested by recent molecular clock estimates (e.g., 100 Ma [25], 90 Ma [26], and 75 Ma [27]). Furthermore, our findings indicate that advanced feather molt strategies, like many other avian characteristics, appeared early in paravian evolution.

Previous studies have suggested that *Microraptor* was only capable of gliding flight [28–31]. The evidence here suggesting a sequential molt strategy strongly supports elaborated aerodynamic abilities, which could consist of either flapping or gliding flight or at least the necessity for flight throughout the entire annual cycle, including during the molting period, for the daily survival of this taxon. As our data from extant birds indicate, sequential molts that allow flight ability to be retained during the molting period likely evolved to maintain escape and movement-dependent foraging capacities throughout the year [13, 14]. Elaborated flight capabilities in *Microraptor* are also supported by the strong degree of asymmetry present in the vanes of its flight feathers and the presence of an alula [19]. The

sequential molt of *Microraptor* may also suggest that the habitats used by this species during the molting period did not provide adequate protection from predation.

Our findings highlight the importance of linking form and function in modern animals in order to facilitate evolutionary inferences in extinct taxa. Such information can shed light on how morphology is shaped by aerodynamic considerations even during a dynamic morphological process that occurs throughout an animal's annual cycle. This work demonstrates the importance of considering biomechanical aspects, morphology, ecology, and annual routine processes in order to improve our understanding of paleontology and organismal evolution [32].

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
 - Lead Contact
 - Materials Availability
 - Data and Code Availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
 - Fossil examination
 - Molt strategy classification
 - Phylogenetic analysis
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.cub.2020.06.046>.

A video abstract is available at <https://doi.org/10.1016/j.cub.2020.06.046#mmc4>.

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AUTHOR CONTRIBUTIONS

Y.K. and A.B. conceived the study. Y.K., A.B., and X.X. tested the fossil. Y.K. performed phylogeny analyses. N.S. supervised the analyses. Y.K. wrote the manuscript. A.B., N.S., J.O., M.W., and X.X. contributed to manuscript writing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and Algorithms		
BirdTree	[15]	http://birdtree.org/
BEAST 1.8.4	BEAST Developers 2020	https://beast.community/2016-06-17_BEAST_v1.8.4_released.html
R 3.6.2	R Development Core Team 2016	https://cran.r-project.org/
R package 'phytools'	[33]	https://cran.r-project.org/web/packages/phytools/index.html

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Yosef Kiat (yosefkiat@gmail.com).

Materials Availability

The specimen of *Microraptor* is stored in the Institute of Vertebrate Paleontology and Paleoanthropology (Beijing, China) under the collection number IVPP V13352. This study did not generate new unique reagents.

Data and Code Availability

The datasets and phylogenetic tree generated during this study and the phylogenetic tree used are available as [Supplemental Information](#) or at OSF: <https://osf.io/3cxfy/>.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The study subject, IVPP V13352, is an almost complete skeleton of *Microraptor* [19]. The specimen was collected in deposits of the Lower Cretaceous Jiufotang Formation (120 Ma) near Chaoyang City in Dapingfang, Chaoyang County, western Liaoning, and was housed in the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP), Beijing, China.

METHOD DETAILS

Fossil examination

We examined the *Microraptor* specimen, IVPP V13352, stored in the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP), Beijing, China. The specimen was visually inspected under appropriate light conditions and photographed. We visually inspected the rachis and barbs of the feathers to identify each of the wing's flight feathers, as well as each feather's stage of growth.

Molt strategy classification

Since feathers cannot be renewed continuously from their base, like other keratinous structures (e.g., hair and claws), they are replaced only after the shedding of old feathers [7]. The time lag between feather shedding and the full growth of the new feather creates a feather gap. Because several adjacent feathers may be shed during a short time interval, feather gaps of various widths and lengths are created during the molt process [7, 9, 34]. The size of the feather gap is determined by the number of feathers that have been shed within a short time interval and by the growth rate of the feathers, with the former factor being more important than the latter [35]. Molt-related feather gaps may substantially hamper flight performance and increase flight metabolism over a period of weeks to months [10, 11, 36–38].

Another factors that may affect the size and shape of molt-related feather gaps is the sequence of feather molting [39, 40]. The most common molt sequence of the primary feathers is outward, starting with the renewal of the innermost primary (P_1), and moving outward toward the distal tip of the wing [7, 9]. Additional sequences that are used during primaries molt are outward sequence from more than a single center (e.g., Common Kingfisher *Alcedo atthis*; [Figure S2](#)), inward and outward molt from a single center (e.g., Peregrine Falcon *Falco peregrinus*) or inward molt (e.g., Spotted Flycatcher *Muscicapa striata*) [8, 9, 39]. All these four primaries molt strategies are characterized by a sequential molt that enables flight during the molting period. In contrast, a minority of bird species molt their primary feathers non-sequentially, either simultaneously or through gradual not ordered molt in which feather replacement takes place without any predictable sequence or direction, and, in the latter case, usually asymmetrically between the two

wings. In order to study the relationship between molt strategy and life-history traits we classified each bird species in this study by its molt pattern, being either “sequential molt” or “non-sequential molt”. “Sequential molt” is characterized by a clear direction of the replacement sequence within the primary feathers and “non-sequential molt” is characterized by the synchronization of the molt of all remiges or by a non-ordered, irregular molt that is identified by the lack of direction in the sequence of feather replacement of the primaries (see examples in [Figure S2](#)). We note that simultaneous molt might have evolved as a result of a rapid sequential molt.

Notably, we identified a flexible molt strategy in two species. In the Great Hornbill (*Buceros bicornis*), females molt non-sequentially (probably simultaneously) during the breeding period when they remain in their nesting cavity during a long time period and cannot fly. Nonetheless, non-breeding females and males of this species exhibit a sequential molt. In the Greater Flamingo (*Phoenicopterus roseus*) that is usually characterized by a non-sequential, simultaneous molt, the non-sequential molt does not take place in some occasions, presumably when the habitat is not safe enough for losing the flight capability, even for a short period [41].

Phylogenetic analysis

The phylogenetic tree ([Figures 1](#) and [S3](#)) was obtained from an analysis of global bird diversity [15] using 10,000 trees that were generated from the BirdTree project (Ericson all species: a set of 10,000 trees with 9,993 OTUs each; <http://BirdTree.org> [42]). The consensus tree was built using BEAST version 1.8.4 (median heights). In order to study the evolution of molt sequence (sequential versus non-sequential molt strategy), as well as for estimating and visualizing the ancestral strategy, we used an ancestral reconstruction trait analysis under a continuous-time Markov chain, using the R package ‘phytools’ (version 0.6-99; Phylogenetic Tools for Comparative Biology) [33]. The analysis included 302 bird species belonging to 31 orders in the class Aves, 112 species with non-sequential molt strategy and 190 species with sequential molt strategy. These species included the following number of species from each order: 36 Anseriformes species, 11 Galliformes species, 14 Pelecaniformes species, 2 Ciconiiformes species, 3 Gaviiformes species, 18 Suliformes species, 18 Sphenisciformes species, 12 Procellariiformes species, 3 Otidiformes species, 25 Gruiformes species, 1 Phoenicopteriformes species, 7 Podicipediformes species, 36 Charadriiformes species, 1 Opisthocomiformes species, 8 Apodiformes species, 4 Caprimulgiformes species, 10 Strigiformes species, 3 Piciformes species, 2 Bucerotiformes species, 10 Coraciiformes species, 5 Accipitriformes species, 6 Falconiformes species, 31 Passeriformes species, 14 Psittaciformes species, 3 Cuculiformes species, 6 Columbiformes species, 2 Tinamiformes species, 4 Apterygiformes species, 4 Casuariiformes species, 2 Rheiformes species and 1 Struthioniformes species ([Table S3](#)). This phylogenetic analysis included all extant flightless bird species and a large collection of species for which we have information about their molt strategy. The information about the birds’ molt strategy and flight ability ([Tables S1](#) and [S3](#)) as well as habitat selection ([Table S2](#)) was collected from published literature [7, 9, 17, 43–49] or using the database of the authors.

QUANTIFICATION AND STATISTICAL ANALYSIS

The analysis was performed using R (version 3.6.2; R Development Core Team 2016).